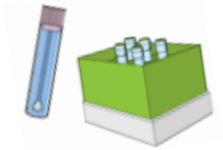
SARS-CoV-2 Sequencing Timeline

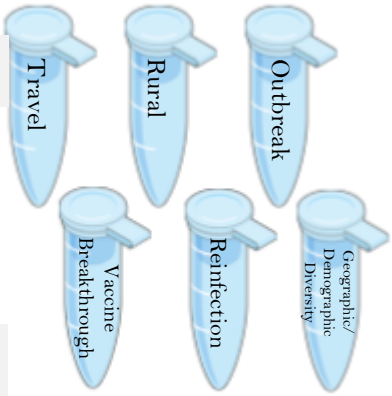
DAY 0: Specimens arrive at Alaska State Virology Laboratory



SARS-CoV-2 specimens submitted for sequencing are accessioned into the sequencing queue upon arrival at ASVL. Specimens submitted for diagnostic or confirmatory SARS-CoV-2 PCR testing will reflex to sequencing after a positive PCR result is obtained.

DAY 1: Specimens are selected and batched for sequencing

Each sequencing batch consists of 46 patient specimens and 2 controls. Specimen selection is based on a priority scheduling algorithm where samples associated with travel, rural areas, known outbreaks, vaccine breakthroughs, and reinfections are batched first. Specimens representative of geographic and demographic diversity are selected to complete the batch.



DAYS 2 & 3: Library Preparation

Extracted RNA

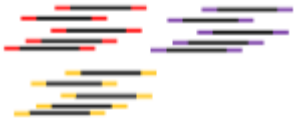
Sequencing libraries are prepared from extracted RNA in a 16-hour process comprised of a series of enzymatic reactions that begins with the conversion of viral RNA to cDNA and ends with the pooling of 48 "libraries" of barcoded DNA fragments generated from each specimen and control in the batch.

QC Check: RNA

Viral RNA is converted to cDNA

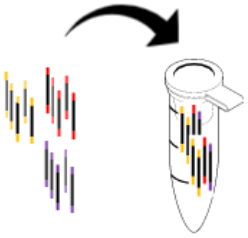
400 base pair amplicons tiling the viral genome are generated via primer-based amplification for SARS-CoV-2 targeted sequencing

QC Check: DNA



Amplicons undergo adaptor ligation and are assigned a unique index that acts as a molecular barcode to distinguish between specimens

QC Check: Libraries



Each indexed library (48 per batch) is combined into a final pool for multiplexed sequencing

QC Check: Final Library Pool

The final library pool is loaded onto the Illumina MiSeq instrument to begin the 56-hour sequencing process

DAYS 3, 4, & 5: Sequencing

Prepared libraries are loaded on a flow cell where adaptors facilitate attachment of DNA fragments to the flow cell surface and clonal amplification begins. The resulting cluster of template DNA undergoes multiple rounds of sequencing to generate millions of reads. A unique fluorescent signal is emitted for each of the four bases (A,G,T,C) which allows the instrument to issue base calls for nucleotides at each location in the sequence.

DAY 6: Genome Assembly and Data Analysis

Fragmented DNA reads are then assembled by aligning sequenced fragments to a reference genome (SARS-CoV-2 isolate Wuhan-Hu-1). Assembled genomes are assessed for quality before variant calls are issued based on nucleotide differences between the sequenced virus and reference.